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Pyralis papaleonei sp. nov. from Crete (Greece) (Lepidoptera, Pyralidae)

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Abstract. *Pyralis papaleonei* **sp. nov.** is described based on diagnostic phenotypic and genital morphological characters as well as unique DNA barcodes (COI gene – cytochrome c oxidase subunit 1) and compared with closely related taxa occurring in the Eastern Mediterranean, namely *P. kacheticalis* and *P. regalis*. The new species is endemic to the island of Crete and currently only known from the White Mountains (Lefka Ori) in the western part of the island. *Pyralis kacheticalis* is recorded from Iraq and Armenia for the first time.

Introduction

European species closely related to *Pyralis regalis* [Denis & Schiffermüller], 1775 have long been popular subjects among entomologists due to their striking colours and wing patterns, and they are therefore represented in many collections. Until a few years ago, however, they were considered largely unproblematic from a taxonomic perspective. The recent descriptions by Leraut (2005) and Wikström et al. (2020) served as a clear reminder that even among such conspicuous European moths, overlooked species remain to be discovered. Most recently, this shortcoming in the basic inventory of the European fauna was underscored by spectacular findings of further enigmatic species, such as *Carcina ingridmariae* Huemer, 2025 (Huemer 2025).

The taxonomy of the *Pyralis regalis* species group, however, seemed more or less resolved—at least superficially—following the discovery and description of *Pyralis cardinalis* Kaila et al., 2020 (Wikström et al. 2020). Attentive readers, however, could already discern doubts about the taxonomic status of certain populations of *Pyralis kacheticalis* (Christoph, 1893) in the context of that description. Genetic data (DNA barcodes) indicated a considerable degree of variation within this taxon which, however, was interpreted as intraspecific. One sequence from Crete was particularly divergent, raising doubts about its assignment to *P. kacheticalis* (Wikström et al. 2020).

Meanwhile, one of the authors (PH), as part of extensive studies on the Lepidoptera fauna of Crete (Huemer et al. 2025), has been able to sample and sequence substantial additional

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material of the doubtful taxon. The molecular data now available, in combination with a thorough morphological re-analysis, confirm the existence of an additional and hitherto unnamed species, which is described below.

Material and methods

In addition to approximately 600 *Pyralis* specimens previously examined by us (Wikström et al. 2020), about 100 specimens from the *Pyralis regalis* species group were analysed, including 45 individuals of the new species and 15 specimens of *P. kacheticalis*. The supplementing material was pinned, dried, and either spread or traditionally set.

Male and female genitalia were prepared following Robinson (1976). Descriptive terminology follows Wikström et al. (2020).

Ninety previously published DNA barcode sequences of the mitochondrial COI gene (658 bp of the 5′ terminus of the cytochrome c oxidase subunit I gene) (Wikström et al. 2020) were supplemented with 38 additional *Pyralis* spp. samples to generate further sequences. DNA tissue samples (dried legs) were processed at the Biodiversity Institute of Ontario (University of Guelph, Canada) following the standard high-throughput protocol of deWaard et al. (2008). Complete voucher data and images are available in the public dataset [DS-PYRALCRE] "New *Pyralis* from Crete" dx.doi.org/10.5883/DS-PYRALCRE in BOLD (Ratnasingham and Hebert 2007; Ratnasingham 2018).

With the exception of the neotype of *P. regalis*, all sequences were assigned to Barcode Index Numbers (BINs), algorithm-based operational taxonomic units that provide an accurate proxy for species (Ratnasingham and Hebert 2013). BINs are automatically calculated in BOLD for records that comply with the DNA barcode standard. Degrees of intra- and interspecific variation in the DNA barcode fragments were calculated under the Kimura 2-parameter model of nucleotide substitution using the analytical tools of BOLD Systems v. 4.0. A neighbor-joining tree of the DNA barcode dataset was constructed using MEGA 11 (Tamura et al. 2021) under the Kimura 2-parameter model.

Photographs of adults were taken with an Olympus OM-D Mark III camera equipped with a 60-mm macro lens, while genitalia images were captured using a Zeiss Axiolab 5 microscope connected to an Olympus OM-D Mark III camera. Image stacking was performed using Helicon Focus 8.0, and final image processing was carried out in Adobe Photoshop 6.0.

The morphological characteristics of species of the *P. regalis* species group are described and illustrated in detail in Wikström et al. (2020) and are available as open access. We therefore illustrate only the species recorded from the eastern Mediterranean region, *P. kacheticalis*, *P. regalis*, as well as the newly described species, and additionally refer to the aforementioned publication.

A linguistic review was conducted on ChatGPT.

Abbreviations of collections

MZH Finnish Museum of Natural History, University of Helsinki, Finland

TLMF Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria

ZMUC Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark

ZSM Staatliche Naturwissenschaftliche Sammlungen Bayerns, Abt. Zoologische Staatssammlung München, Munich, Germany

Results

Checklist of species in the *Pyralis regalis* species group

Pyralis regalis [Denis & Schiffermüller], 1775
= *Pyralis pulchellalis* Millière, 1873
Pyralis sagarrai Leraut, 2005
Pyralis kacheticalis (Christoph, 1893)
= *Pyralis imperialis* Caradja, 1916
Pyralis papaleonei sp. nov.
Pyralis cardinalis Kaila, Huemer, Mutanen, Tyllinen & Wikström, 2020
= *Pyralis subregalis* Caradja, 1926 *nec* Snellen, 1895
Pyralis princeps Butler, 1889
Pyralis joannisi Leraut, 2005
Pyralis perversalis (Herrich-Schäffer, 1849)

Molecular analysis

DNA sequencing resulted in nine BINs, corresponding to all known species of the *Pyralis regalis* species-group except for *P. joannisi* with unknown barcode and two unnamed clusters from China. Intraspecific variation is low, with p-distances ranging from 0% to 2.82%, with the exception of *P. kacheticalis* with a geographically linked mean intraspecific variation of 2.16% and maximum of 3.81%, and *P. regalis* with a maximum divergence of 2.82%. A considerable intraspecific variation is also documented for the congeneric European species *P. farinalis* (Linnaeus, 1758) and *P. lienigialis* (Zeller, 1843) (Table 1). Interspecific distances to the nearest species are substantially higher, ranging from 4.1% to 8.82% within the *P. regalis* species group and up to 10.86% considering the above mentioned congeners (Table 1, Fig. 1).

Table 1. Intraspecific mean K2P (Kimura 2 Parameter) divergences, maximum pairwise distances, BIN, number of BIN members (n), nearest species and distance to NN (Nearest Neighbor) (distances in %) in European species of *Pyralis*, based on BOLD barcode gap analysis from sequences in dataset.

Species	Mean Intra-Sp	Max Intra-Sp	BIN	n	Nearest Species	Distance to NN
Pyralis regalis	0.49	2.82	BOLD:AAP5668	46	*Pyralis sagarrai*	4.1
Pyralis sagarrai	0.18	0.31	BOLD:ADS1090	3	*Pyralis regalis*	4.1
Pyralis cardinalis	0.32	1.18	BOLD:AAF5806	29	*Pyralis kacheticalis*	7.92
Pyralis kacheticalis	2.16	3.81	BOLD:AEB9019	1	*Pyralis papaleonei*	5.78
			BOLD:ABA8506	4	sp. nov.	
			BOLD:ABA9291	6		
Pyralis papaleonei sp. nov.	0	0	BOLD:AAY9338	5	*Pyralis kacheticalis*	5.78
Pyralis princeps	0	0	BOLD:ABA8505	2	*Pyralis kacheticalis*	8.82
Pyralis perversalis	0	0	BOLD:AAX9987	2	*Pyralis kacheticalis*	6.97
Pyralis farinalis	0.97	5.74	BOLD:AAY8728	19	*Pyralis kacheticalis*	10.83
			BOLD:AAB3316	2		
Pyralis lienigialis	1	2.5	BOLD:AAF5809		*Pyralis farinalis*	10.86
			BOLD:ADK8626			

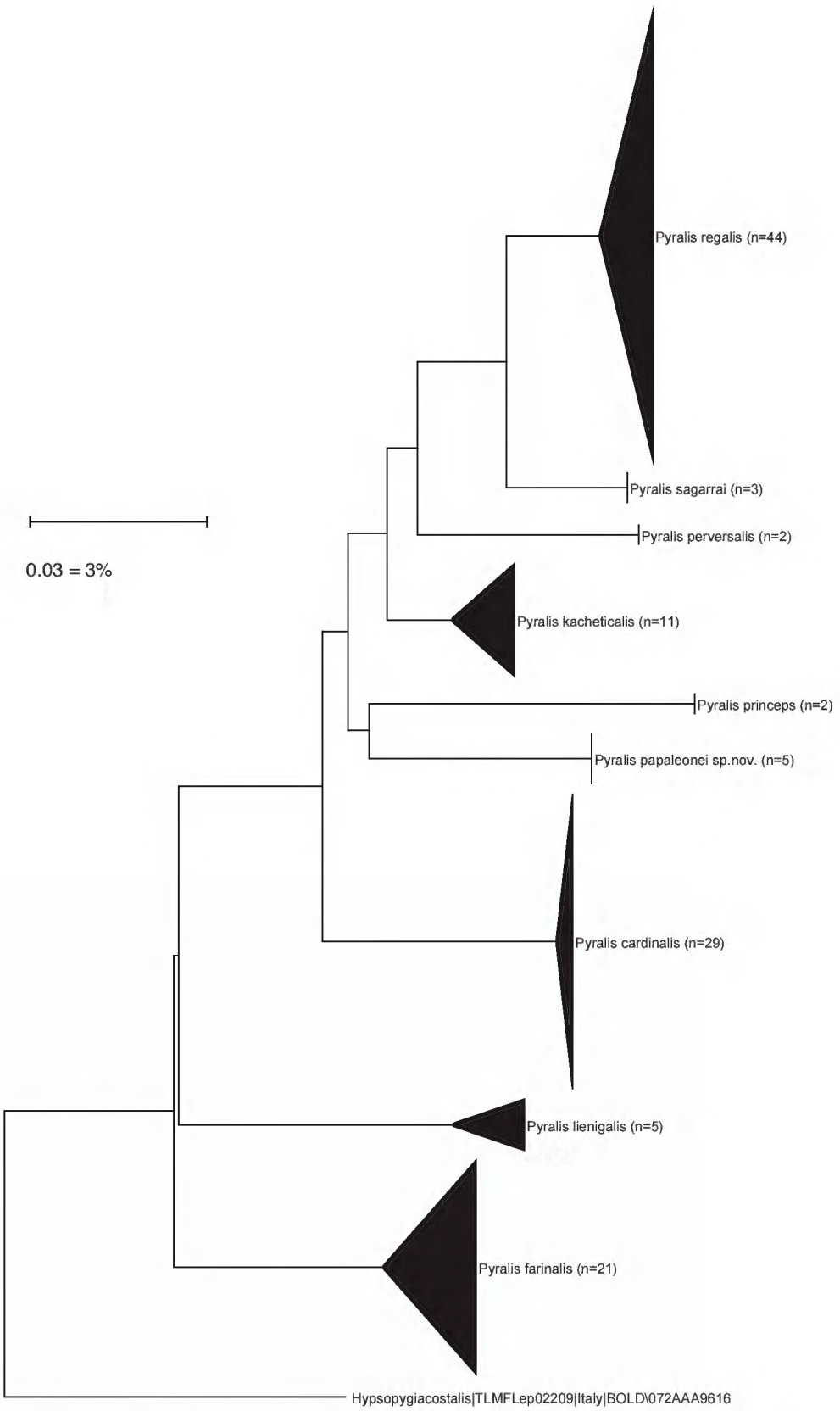


Figure 1. Neighbor-Joining tree of Euroepean *Pyralis* spp. (Kimura 2-parameter, built with MEGA 11 (Tamura et al. 2021); Source: DNA barcode data from BOLD (Barcode of Life Database; Ratnasingham 2018); rooted for *Hypsopygia costalis* as outgroup. Note: the scale bar only applies to internal branches between species. The width of the triangles represents the sample size, and the depth the relative genetic variation within the cluster (2× scale bar).

Two additional BINs of alleged *P. regalis* from China based on public sequences most likely belong to undescribed species. Externally these taxa seem to correspond to the *Pyralis regalis* species group. BIN: BOLD: AAL8568 is 4.13% distant from *Pyralis cardinalis*, whereas BIN: BOLD: AEG1231 is 7.53% distant to *Hypsopygia cohortalis* (Grote, 1878) from North America. Both BINs are not considered in Table 1 but listed in the dataset.

Pyralis papaleonei sp. nov.

https://zoobank.org/447F3F9E-9DAD-419C-8C78-D29FA775DCFF

Figs 2, 3, 6, 9, 12, 13

Holotype. Greece • ♂; Crete, Omalos, Mikes House; 1075 m; 35°20'04"N, 23°53'03"E; 18 June 2025; leg. Huemer; TLMF.

Paratypes. Greece • 8 ♂; same data as holotype; gen. slide PYR 390 P. Huemer; TLMF • 11 ♂, same data, 20 June 2025; DNA Barcodes TLMF Lep 46643, 46444, 46645, 46646; TLMF • 2 ♂; Crete, Lakki, 4.2 km SSW; 920–930 m; 35°22'00"N, 23°54'52"E; 19 June 2025; leg. Huemer; TLMF • 9 ♂, 2 ♀; Crete, Omalos plateau, 1 km Kalergi; 1225 m; 35.3202°N, 23.9215°E; 15–20 June 2014; leg. C. Hviid, O. Karsholt & F. Vilhelmsen; ZMUC • 7 ♂; Crete, Omalos plateau; 1040 m; 35.3443°N, 24.9048°E; 14–20 June 2014; leg. C. Hviid, O. Karsholt & F. Vilhelmsen; ZMUC • 1 ♂, 1 ♀; Crete, 1.7 km S Topolia; 1040 m; 35.2442°N, 23.8054°E; 14–19 June 2014; leg. C. Hviid, O. Karsholt & F. Vilhelmsen; gen. slide Kaila 6311; ZMUC • 1 ♀; Crete, 10.5 km W Omalos plateau; 760 m; 35.3489°N, 23.8154°E; 18 June 2014; leg. C. Hviid, O. Karsholt & F. Vilhelmsen; ZMUC • 1 ♂; Crete, Fassatal; 270 m; 35°24'N, 23°53'E; 11 October 1972; leg. H. Malicky; TLMF • 1 ♂; Crete, Omalos-Hotel; 1041 m ["1000 m"]; 35.34429 N; 23.9046 E; 05 July 2010; DNA Barcode BC ZSM Lep 46538; leg. W. Ruckdeschel; ZSM.

Diagnosis. *Pyralis papaleonei* sp. nov. is distinguished from all congeners by its narrow white forewing markings, featuring an almost uniformly slender white median band extending across the entire wing (Figs 2, 3). In all other species of the group (Figs 4, 5), except for specimens of *P. kacheticalis* from Cyprus, the white pattern elements are distinctly broader, and in some cases abruptly narrowed medially or terminating in the middle of the wing. Also characteristic are the nearly uniform pale greyish-purple hindwings, which are divided into three sections by two white lines; the basal section is only slightly darker than the outer sections. In contrast, in *P. kacheticalis* (Fig. 4) and *P. sagarrai* particularly the median and also the outer sections of the hindwings are much lighter than the remaining areas, while the basal section is conspicuously darkened and clearly demarcated. The hindwings of *P. regalis* (Fig. 5) and *P. cardinalis*, which are likewise slightly darker overall, are densely suffused with a light purplish tinge of variable intensity, especially the marginal area. Moreover, the white pattern on the forewings of all these three species is broader. Finally, *P. princeps* and *P. joannisi* are easily distinguished from the new species by features such as forewing with pointed apex and with a much darker ground colour, extensive purple suffusion along the fore- and hindwing margins, and conspicuously broad and short white streaks of the forewing (see Wikström et al. 2020).

The male genitalia of *P. papaleonei* sp. nov. (Fig. 6) are very similar to those of the other species of the *P. regalis* species group. They differ from *P. cardinalis*, *P. kacheticalis* (Fig. 7) and *P. princeps* by the absence of cornuti in the phallus, from *P. regalis* (Fig. 8) and *P. perversalis* by the shape of the valva without abruptly widened distal part, and from *P. joannisi* by the smooth basal part of the juxta, without spines. The dense group of elongate spine groups in the vesica of *P. papaleonei*, reminiscent of that in the Oriental species *P. princeps* and *P. joannisi* (Wikström et al. 2020).

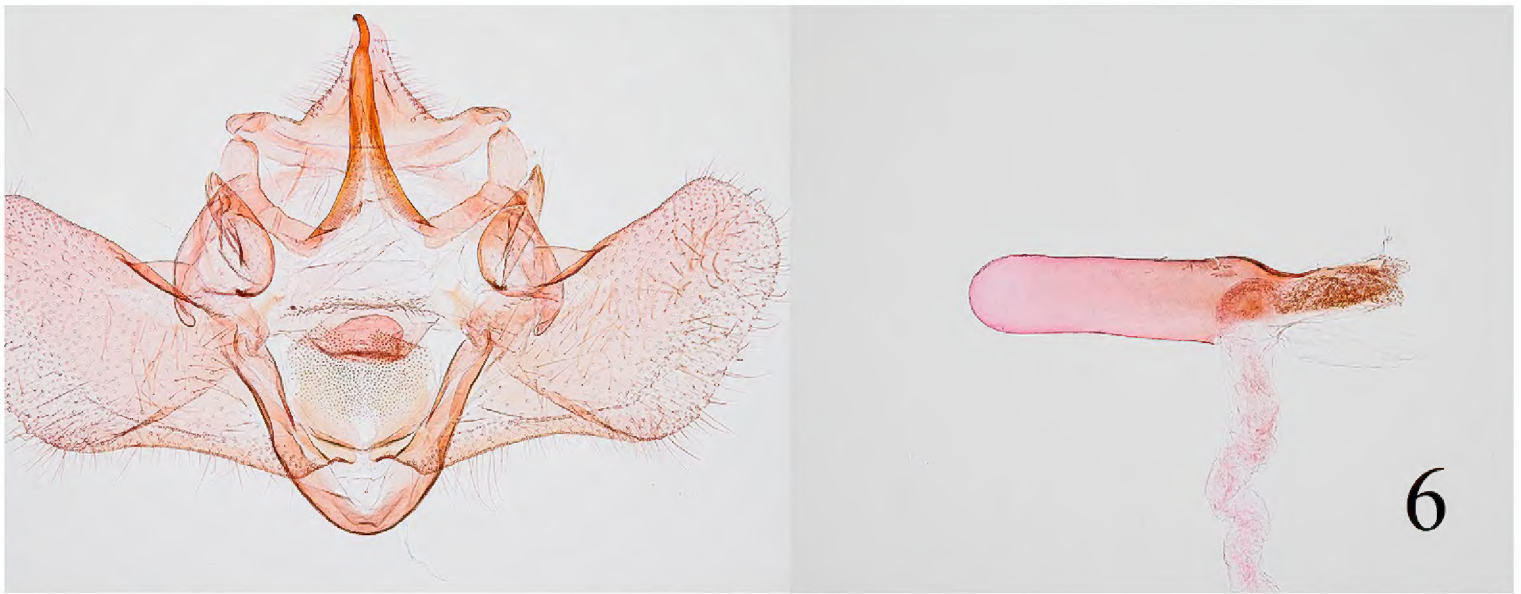
Female genitalia of *P. papaleonei* sp. nov. differ from all other species of the *P. regalis* species group by the presence of a sclerotized signum, absent in other species, and by the shape of the sterigma with broadly rounded posterior projections (Figs 9–15).

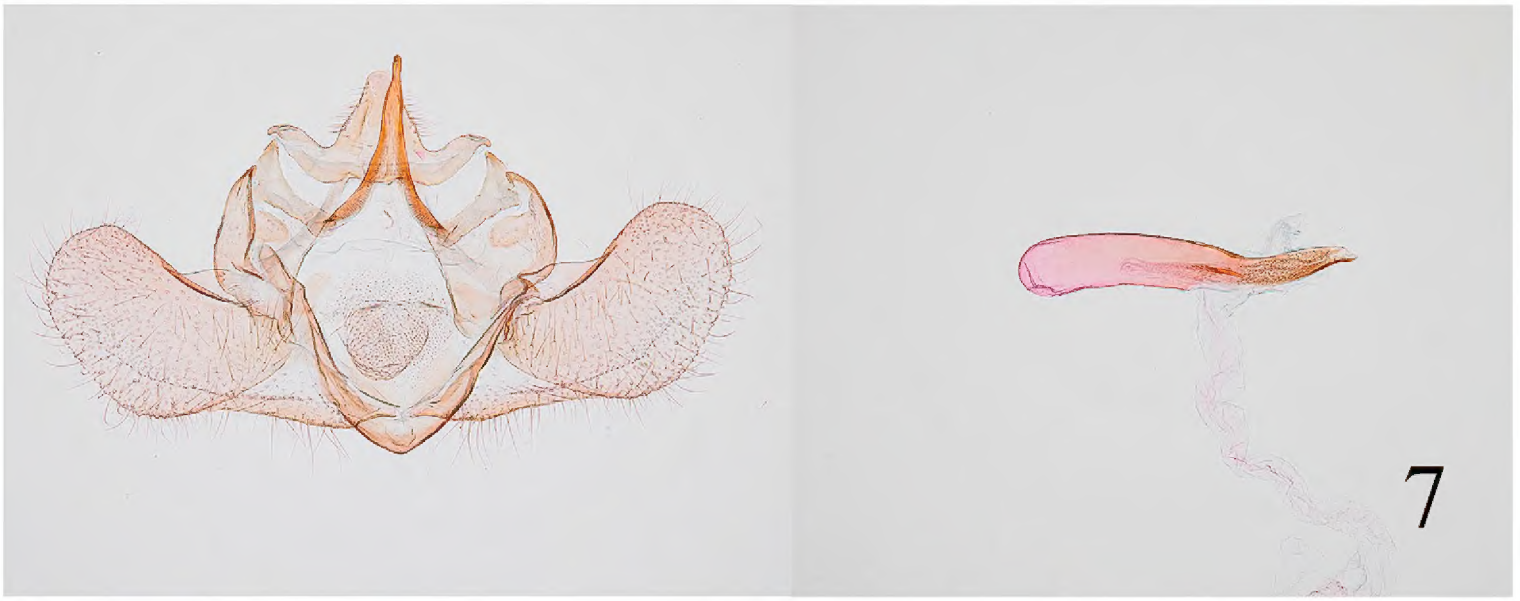
Description. Adult (Figs 2, 3). Forewing length: ♂: 7.5–9.4 mm, ♀: 10.0 mm. Proboscis well developed, basally scaled; labial palpus ascending, second segment about 2.5 times as long as third segment; maxillary palpus short, about one-quarter length of labial palpus; head rough-scaled;

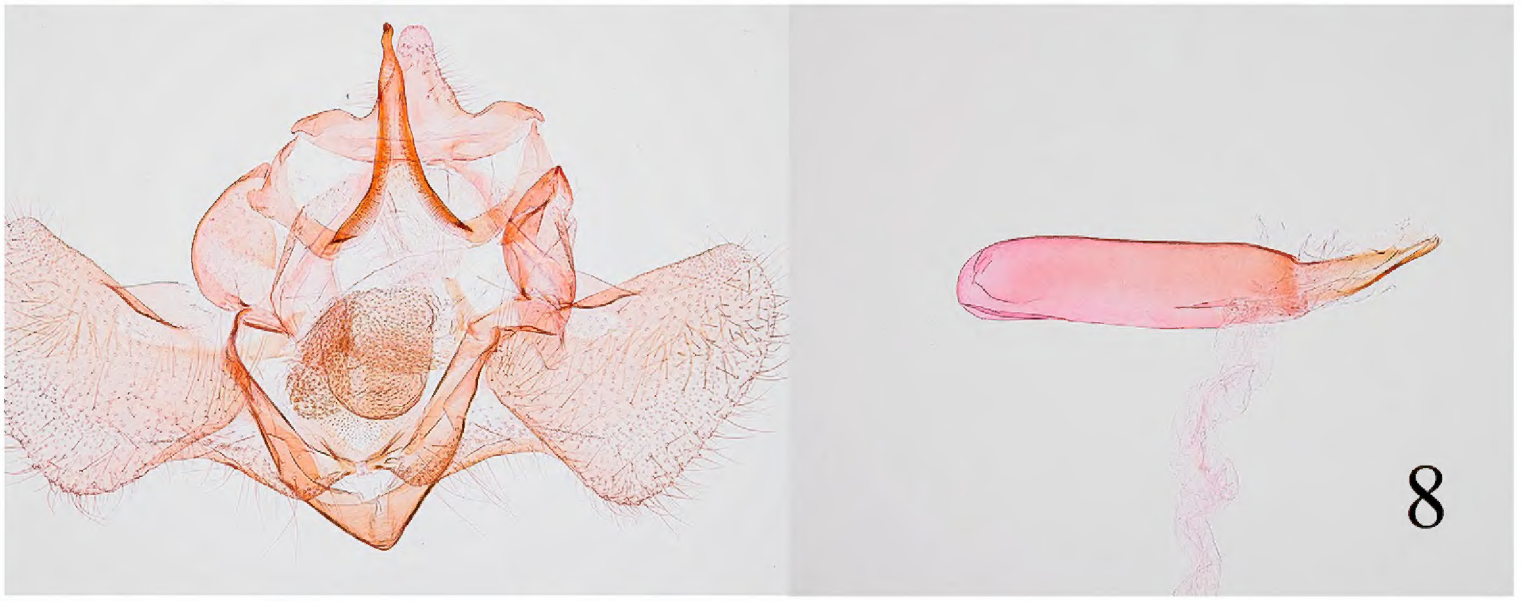
Figures 2–5. *Pyralis* spp., habitus. **2.** *P. papaleonei* sp. nov., holotype, ♂; **3.** *P. papaleonei* sp. nov., paratype, ♂; **4.** *P. kacheticalis*, ♂, Georgia; **5.** *P. regalis* ♂, Italy (TLMF).

male flagellum ciliate, length of each cilium twice diameter of shaft; female antenna with very short cilia; head and all appendices yellow with some purple, except for purely purple flagellum. Thorax and dorsal surface of abdomen purple, ventrum yellowish mixed with some purple. Legs yellowish, forelegs and midlegs mixed with purple on outer surface.

Forewing ground colour purplish, costal area predominantly reddish brown, in medial third with orange tinge; narrow and weakly curved white fascia from costa to dorsum, last third towards fold slightly tapered; short white streak from costa at four-fifths of wing of about one-third length of wing width, with inconspicuous and barely perceptible whitish line towards dorsum; several small white or orange-white spots along costa between white markings; discal area of forewing with large orange patch; fringe purple-brown with indistinct fringe line. Hindwing almost unicolorous pale greyish-purple, divided into three sections by two irregularly jagged whitish lines; basal section at most minimally darker than outer sections; fringe purple-brown with indistinct fringe line. Forewing underside pale purple-brown, costal area predominantly ochre-brown, with several yellowish spots in basal half, and yellowish-ochre streak corresponding to outer white area of upper side; hindwing underside purple-brown with predominantly ochre-brown costal area andcurved pale-ochre line along outer two-thirds.





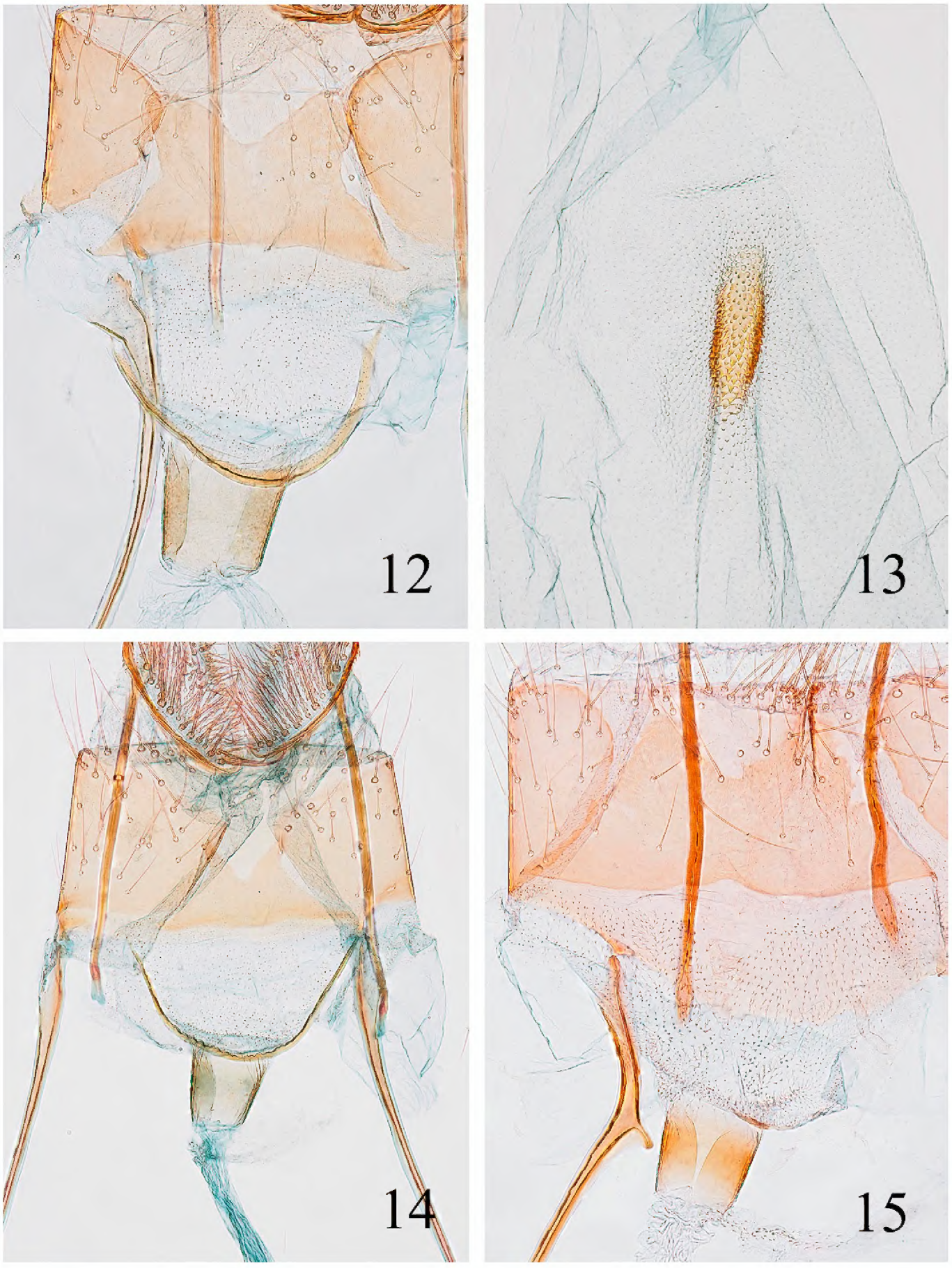


Figures 6–8. *Pyralis* spp., male genitalia. **6.** *P. papaleonei* sp. nov., paratype, ♂, gen. slide PYR 390 P. Huemer, Greece (TLMF); **7.** *P. kacheticalis*, ♂, gen. slide 25/1555 P. Huemer, Georgia (MZH); **8.** *P. regalis* ♂, gen. slide PYR 392 P. Huemer, Italy (TLMF).

Male genitalia (Fig. 6). Uncus hat-shaped, anterior edge convex, posteriomedial part sub-triangular with blunt-tipped apex, laterally sparsely setose. Tegumen narrow, band-like. Gnathos arms articulated from tegumen, basally widened, distal part rod-shaped, mesially a narrow, triangular

Figures 9–11. *Pyralis* spp., female genitalia. **9.** *P. papaleonei* sp. nov., paratype, gen. slide 25/1553 P. Huemer, Greece (ZMUC); **10.** *P. kacheticalis*, ♂, gen. slide 25/1555 P. Huemer, Georgia (MZH); **5.** *P. regalis*, PYR 391 P. Huemer, Italy (TLMF).

lobe terminating with abruptly bent, fused hook-shaped apex; median part of gnathos about twice as long as uncus. Valva about twice as long as wide; ventral margin weakly concave; valva slightly widened medially and ending in an increasingly narrowed, apically broadly rounded cucullus; costa straight, weakly sclerotized, dorsoproximal margin of valva with sclerotized connection to vinculum. Transtilla laterally shortly sclerotized, median part membranous, weakly spinose. Juxta broadly tongue-shaped, scobinate. Anellus membranous. Vinculum moderately long, weakly v-shaped with rounded apex. Phallus rod-like, about five times as long as maximum width; basally rounded, cylindrical, apical third narrowed. Vesica weakly spinose, without cornuti.



Figures 12–15. *Pyralis* spp., details of female genitalia. **12.** *P. papaleonei* sp. nov., segment VIII, paratype, gen. slide 25/1553 P. Huemer, Greece (ZMUC); **13.** Ditto, signum; **14.** *P. kacheticalis*, gen. slide 25/1554 P. Huemer, Georgia (MZH); **15.** *P. regalis*, gen. slide PYR 391 P. Huemer, Italy (TLMF).

Female genitalia (Figs 9, 12, 13). Papilla analis dorsally fused, anteriorly widened, apically broad, rounded, ventral part densely setose. Apophysis posterioris about as long as papilla analis. Apophysis anterioris about 1.5 times as long as apophysis posterioris.

Segment VIII dorsally smoothly sclerotized, ventrolateral part with concave edge, with membranous separation from large ventromedial sterigma; sterigma plate-like, posterior edge medially emarginated with large sub-oval lateral projections, lateral edge excavated and obliquely directed outwards, with two pointed terminal extensions, anterior edge weakly convex. Ostium bursae anteriad of sterigma, membranous, covered with microtrichia, anteriorly demarcated by distinctly sclerotized, sub-oval ridge, antrum absent. Entrance of ductus bursae with dorsolaterally distinctly sclerotized, weakly funnel-shaped colliculum. Ductus seminalis incepted anterior to colliculum, without swelling or sclerotization; posterior half of ductus bursae braid-shaped, granulose, otherwise membranous, evenly widening towards corpus bursae and fusing into it. Corpus bursae elongate, oval, with large field of weakly sclerotized microthorns; lens-shaped signum well developed with strongly sclerotized microthorns.

Biology. Host-plant and early stages are unknown. Adults have been collected at light mostly in June with a single specimen in October, indicating a long flight season, or possibly a second generation. The species occurs in mountainous habitats with the vast majority of material collected at about 1000 to 1200 m (Fig. 16). No observation was made near the coast.

Molecular data. BIN: BOLD:AAY9338. The minimum distance to the nearest neighbour, *P. kacheticalis* populations from Greece (Samos), Ukraine, Armenia and Iraq is 5.78% (BIN: BOLD:ABA9291), whereas the minimum distance to *P. regalis* is 9.4% (BIN: BOLD:AAP5668).

Distribution. Only known from few localities in the White mountains in western Crete.

Etymology. The new species is dedicated to the head of the Catholic Church, Pope Leo XIV. The Pontiff is a strong advocate of climate and environmental protection, and we hope that his voice may serve as an example for humanity. Furthermore, due to its distinctive coloration and overall

Figure 16. Type-locality of *P. papaleonei* sp. nov. (Greece, Crete, Omalos plateau).

appearance, the new species belongs to a group of Pyralidae whose species names refer to high secular or ecclesiastical offices, including *Pyralis regalis*, *Pyralis imperialis* (= *P. kacheticalis*), *Pyralis princeps*, and *Pyralis cardinalis*.

Remarks. New country records of *P. kacheticalis*: ARMENIA • 4 ♂; Prov. Wajoz Dsor, Areni, Noravan road; 1240 m; 39.667951°N, 45.221408°E; 1–4 July 2024; leg. Rachinger; TLMF; • 1 ♂; same data, but 29 September 2024; TLMF. IRAQ • 1 ♂; Sulaimaniyya, Dukan, Zarzi village; 660 m; 35.811°N, 45.317°E; 28 June 2024; leg. Khudur; TLMF.

Discussion

The discovery of a new endemic Lepidoptera species on Crete fits seamlessly into the series of species descriptions that have been published from the island in the last few decades. Indeed, 40% of the currently documented 76 endemic species of the island have been described since the turn of the millennium (Huemer et al. 2025). Nevertheless, the new species is surprising—especially considering that in the nearest regions of Greece (Peloponnese) and Cyprus two other members of the species group, respectively, *P. regalis* and *P. kacheticalis*, are present. This may partly explain why *P. papaleonei* sp. nov. has so far been overlooked and was initially misidentified in BOLD as *P. kacheticalis*. Furthermore, the species appears to occur mainly in the less intensively sampled mountain regions of Crete, and has therefore been collected by only a few entomologists.

The new species shares its primary habitat with several other endemics that are, according to current knowledge, also very locally distributed, including *Herrichia vilhelmseniella* Karsholt & Lvovsky, 2018 (Oecophoridae), *Teleiodes albiluculella* Huemer & Karsholt, 2001 (Gelechiidae), *Peribatodes omalensis* Rajaei, Skou & Hausmann, 2019 (Geometridae), and *Cryphia omalosi* Fibiger & Svendsen, 1998 (Noctuidae). Additional undescribed species, such as an *Orectis* sp. (Erebidae), have also been recorded in the greater Omalos region.

Pyralis papaleonei sp. nov. displays an allopatric distribution pattern relative to the other taxa and is the sole representative of the species group on Crete. The considerable genetic divergences of 9.4% (p-dist) from the geographically closest species, *Pyralis regalis* from the Peloponnese, and 6.29% from the Cypriot cluster of *P. kacheticalis*, strongly support its species status when combined with diagnostic morphological features. The nearest neighbour, with 5.78% divergence, belongs to the *P. kacheticalis* cluster distributed from Samos through Armenia and Georgia to Iraq. Current knowledge indicates that members of the *Pyralis regalis* group on the Palaearctic mainland are, aside from minor range overlaps, largely allopatrically distributed. Despite often wide distributions—*P. cardinalis* even being transpalaearctic—intraspecific variability is low in some species but considerable with c. 2% divergence in others.

Therefore, in addition to the new species described herein, the *Pyralis regalis* species group appears to comprise additional taxonomically problematic taxa that require clarification. *Pyralis kacheticalis* exhibits pronounced, geographically correlated divergence in DNA barcode sequences, which seems partially accompanied by corresponding morphological differentiation. Moreover, two genetically distinct and isolated clusters from China, misidentified as *P. regalis*, presumably represent undescribed species. Comparable patterns of putatively intraspecific DNA barcode variation have also been reported in other congeners, including *Pyralis lienigialis* (Zeller, 1843) and *Pyralis farinalis*. Integrative taxonomic approaches will be required to resolve their taxonomic status.

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